

Previews

Long Life: A Matter of Taste (and Smell)

Insulin/IGF signaling has emerged as a central regulator of metazoan aging. In *C. elegans*, insulin-like peptides are expressed predominately in neurons. Alcedo and Kenyon demonstrate that removal of specific gustatory and olfactory neurons result in longer life, suggesting that metazoan longevity is influenced by sensory perception.

A breathtaking feat of the nervous system is the seamless integration of incoming sensory information with the demands of internal physiology. Neuroendocrine systems are particularly adept at coupling signals such as nutrient availability to the intricate orchestration of metabolism, growth, and maturation, ensuring maximal survival and reproductive success. Such mechanisms originated early in primitive metazoans.

Despite its anatomical simplicity, the worm *C. elegans* has evolved a remarkably refined endocrine system that controls metabolism, development, behavior, and life span. Genetic studies in *C. elegans* originally revealed that insulin/IGF signal transduction dramatically regulates nematode longevity (reviewed in Tatar et al., 2003). Weak mutations in the insulin/IGF receptor, *daf-2*, and *age-1*/phosphatidylinositol-3-OH kinase produce a 2- to 3-fold increase in adult longevity, with little compromise in fertility and vitality, and this extension depends on the FOXO transcription factor, *daf-16*. The prevailing view is that in favorable conditions, insulin-like agonists acting through the receptor tyrosine kinase activate the PI3 kinase and trigger a kinase cascade that results in the phosphorylation of DAF-16 and its nuclear exclusion. When animals sense environmental stress, the pathway is downregulated, sending DAF-16 into the nucleus where it promotes programs of somatic endurance and longevity (Tatar et al., 2003).

Importantly, not only are the molecular pathways conserved across taxa, but some of their physiological roles are as well. Notably, mutations that attenuate insulin/IGF signaling in *Drosophila* extend adult life span 80%, while IGF-1 receptor heterozygous (+/–) knockout mice live 33% longer (Tatar et al., 2003). To date, few other pathways so visibly affect life span. Insulin/IGF signaling may have generally evolved to couple environmental stress to somatic endurance. In poor times, the organism shunts resources into survival to outlast adversity, while in good times resources are channeled into growth and reproduction.

But how does all this take place? What environmental and physiological inputs influence insulin/IGF production and secretion? What cells produce the insulin-like peptides (ILPs)? What cells receive the insulin signals? How are these signals integrated into organ or organ-

mal choices? What aspects are cell intrinsic or systemic?

Molecular genetics has been one approach to unraveling these questions. Incredibly, there are 38 ILPs encoded in the worm genome, but only one ancestral receptor (Li et al., 2003; Pierce et al., 2001). Given these combinatorics, *daf-2*/Insulin/IGF receptor mutants are, not surprisingly, pleiotropic and among other traits show increased fat storage, stress resistance, decreased fertility, and constitutive entry into a long-lived alternate larval diapause stage called the dauer.

A handful of ILPs have been functionally analyzed and influence dauer and aging. Interestingly, while some are receptor agonists, others are antagonists (Pierce et al., 2001). Most reside in the major sensory neurons, including the amphid and inner labial organs of the head (Figure 1), but also in nonneuronal tissues such as muscle, intestine, and epidermis. Notably, one neural ILP, *daf-28*, is clearly regulated by sensory cues as food and dauer pheromone, an indicator of population density, revealing an intimate link to nutrient sensing (Li et al., 2003). Further dissection of the ILPs should help sort out their specific physiology.

Another fruitful approach has been to define the cells, tissues, and organs that influence organismal aging by removing their function. Because the position and identity of every cell is known in *C. elegans*, individual cells can be reproducibly ablated by focusing a laser microbeam on them through a microscope. In addition, well-defined mutants that selectively impair or delete certain cells can be used. Such studies in *C. elegans* have shown that neurosensory organs as well as specific gonadal cells influence life span (Apfeld and Kenyon, 1999; Arantes-Oliveira et al., 2002). In particular, mutations that disrupt the structure and function of sensory neurons result in increased longevity, which largely depends on DAF-16 (Apfeld and Kenyon, 1999). Remarkably, laser ablation of the amphid sheath, a glial-like cell that supports the amphid neurons, extends life span as well. Such animals have relatively normal feeding behavior, and thus the sensory perception of the environment appears critical. Because sensory neurons produce ILPs, a simple hypothesis is that disruption of insulin-producing cells would result in attenuated insulin signaling and hence increased life span.

Accordingly, reduced insulin-like signaling also promotes entry into the dauer diapause. Elegant cell ablation experiments had established that a subset of chemosensory amphid neurons regulate dauer formation (Bargmann and Horvitz, 1991; Schackwitz et al., 1996). Namely, ADF, ASI, and ASG prevent dauer, while ASJ, ASK, and ADL promote dauer entry (Figure 1).

In the study reported here (Alcedo and Kenyon, 2004), Alcedo and Kenyon asked whether these gustatory, as well as olfactory, sensory neurons also regulate life span, by performing laser microsurgery experiments. What they discovered is that the neuroendocrine architecture regulating longevity is unexpectedly complex. First, they found by removing the amphid gustatory neurons ASI or ASG, animals lived longer, while ADF had

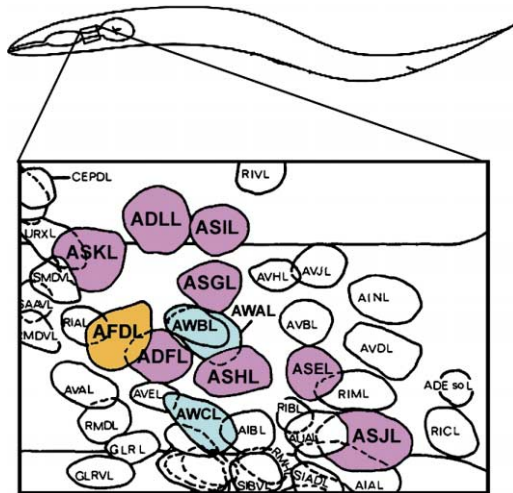


Figure 1. Schematic of *C. elegans* Head Neurons, Left Lateral Aspect

There are 12 bilaterally symmetric amphid sensory neurons. Eight (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, and ASK) are chemosensory (purple), three (AWA, AWB, and AWC) are olfactory (blue), and one (AFD) is thermosensory (orange). (Adapted from the WormAtlas, <http://www.wormatlas.org>, with permission.)

no effect. However, when other gustatory neurons (ASK, ASJ) were additionally removed, longevity was abolished. These cellular epistasis studies suggest that specific cells produce signals that antagonize long life, while other promote it. It is unclear whether they act in parallel or in a linear pathway. A simple model is that ASI/ASG produce an insulin-like agonist; the other cells, an antagonist. Alternately, synaptic transmission from ASI/ASG to ASK could inhibit secretion of an antagonist.

So if sensory signals extend life, what are the relevant cues? The most important factor favoring dauer is elevated levels of dauer pheromone, a mixture of hydroxylated fatty or bile acids (Riddle and Albert, 1997). High temperature as well as dwindling bacterial food also bias dauer. Surprisingly, Alcedo and Kenyon found that dauer pheromone had no effect on life span. This nicely illustrates that distinct cues regulate dauer and aging and that these processes can be uncoupled. It was already known that elevated temperature shortens life span. This leaves the presence or absence of food, or an unknown cue, as the likely culprit. For dauer regulation, the critical component is thought to be carbohydrates or nucleosides, but its identity is not yet known (Riddle and Albert, 1997). A major quest will be to determine whether there are specific metabolites that promote longevity.

How might such cues be detected? Interestingly, reduction of function of the G protein-coupled receptor, *str-2*, as well as $G\alpha$ signaling, modestly extended life span (Alcedo and Kenyon, 2004), revealing potential transducers of sensory cues to insulin-like release. Clearly, elucidating the signal transduction pathway leading to secretion will be an important area of future study. Moreover, *C. elegans* sensory neurons are multifunctional, and sampling internal physiological states may be just as important as the environmental milieu.

C. elegans also possesses three pairs of amphid olfactory neurons (AWA, AWB, AWC) that sense volatile compounds. Alcedo and Kenyon showed that the double ablation of AWA and AWC (but not AWB) extended life span. Moreover, mutants that disrupt their differentiation or function led to a similar increase. Again, perception rather than ingestion is likely at work here. By inference, specific odorants may influence life span itself. When both gustatory and olfactory neurons were removed, correspondingly greater longevity resulted. Notably, the amphid neurons studied here account for about one-sixth of the increase in longevity seen in *daf-2/InsR* mutants, implying that other cells must also contribute.

Olfactory neurons apparently regulate aging somewhat differently than gustatory counterparts. First, induced longevity was not dependent on ASK and ASJ neurons and only partly dependent on *daf-16* (Alcedo and Kenyon, 2004). Importantly, the latter observation suggests that other endocrine signals act parallel or downstream of insulin/IGF signaling to regulate aging. Second, olfactory but not gustatory neurons were shown to interact with signals from the gonad that influence life span. Previous work had suggested that germline stem cells normally produce a signal that antagonizes longevity, while the somatic gonad produces a signal that promotes it (Arantes-Oliveira et al., 2002). Work by Alcedo and Kenyon here implies that part of the somatic gonad effect somehow resides in olfactory neurons. These unexpected connections may reflect complex interactions between gonadal and neural endocrines.

So why has the simple nematode evolved such complexity? Simply put, it is a matter of life or death. Reproductive fitness depends on assessing a complex array of sensory information in diverse environments and making calculated decisions: to disperse or feed communally, to wait out the bad times or commit to reproduction, to couple food cues to reproductive output. Presumably, the relative concentrations of different exogenous substances are predictive for reproductive success, and each nematode must use this information to its advantage. Finally, internal states of different tissues, especially those of the germ cells, must be coordinated throughout the body so that they mature and age at similar rates.

The really exciting question is whether sensory inputs impact neural insulin/IGF signaling in vertebrates and whether the physiological output influences life span. Regions involved in rabbit olfaction and the limbic system synthesize insulins (Devaskar et al., 1994), and humans are reported to produce insulin in response to the smell of food (Brand et al., 1982). In mammals, olfactory neurons also sense molecules signaling territory, dominance, mating receptivity, and other complex traits. In principle, ILPs could mediate some of the immediate metabolic and behavioral responses but might also exert long-term changes on growth, reproduction, maternal effects, and life span. Dietary restriction extends life span in a wide variety of species. Potentially, sensory perception may be an unrecognized aspect of this phenomenon. Thus, healthy aging may require more than healthy diet and exercise. A whiff of those French fries may have unanticipated consequences.

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Selected Reading

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Splicing It Up: A Variant of the N-Type Calcium Channel Specific for Pain

How would you make a drug that inhibits pain without side effects? The most obvious strategy for analgesia targets molecules that are expressed only on neurons used for pain. In this issue of *Neuron*, Bell et al. report a new splice variant of a calcium channel that controls neurotransmitter release and show that it is expressed primarily on nociceptors, the sensory neurons that trigger pain.

Inhibiting N-type calcium channels inhibits pain. The N channel (more properly known as $Ca_v2.2$; Ertel et al., 2000) is the target of morphine on sensory neurons, and injecting selective peptide blockers of N channels into or around the spinal cord greatly relieves pain in animals and in humans (Scott et al., 2002). Mice that lack the N channel gene respond less to noxious stimuli than do wild-type, but they appear otherwise normal (Saegusa et al., 2001; Hatakeyama et al., 2001). However, the peptide blockers can cause severe side effects (Penn and Paice, 2000), demonstrating that it is dangerous to universally block all N channels. Making a better N channel blocker is one of the major tactics for making a better analgesic.

N channels control neurotransmitter release from peripheral neurons, both sensory and autonomic. N chan-

nels are present at synapses in the central nervous system, but neurotransmitter release from most central synapses is controlled more by P/Q-type ($Ca_v2.1$) or R-type ($Ca_v2.3$) calcium channels (Meir et al., 1999). Completing the calcium channel alphabet, L channels ($Ca_v1.x$) control muscle excitation-contraction coupling and neuronal excitation-transcription coupling; T channels ($Ca_v3.x$) have unique electrical properties that contribute to repetitive firing of neurons and pacing of the heart. The interest in N channel blockers as analgesics arises because they are particularly important to sensory neurons. But how to use them to inhibit only pain and no other sensation and to do so without affecting autonomic function? Herein lies the fascination of a nociceptor-specific N channel.

Alternative splicing of calcium channels has been explored systematically before (Lipscombe et al., 2002), but no previous splice variant appeared to be special for pain. That said, previous molecular studies had the weakness that they tested expression in whole dorsal root ganglia, which contains sensory neurons for all the somatic sensations. The power of Bell et al. (2004) is that it explores function and expression of calcium channel subtypes on individual sensory neurons, specifically distinguishing those neurons likely to be nociceptors (pain-sensing neurons). The criteria used to identify nociceptors are sensitivity to capsaicin, the spicy component of pepper that activates thermal nociceptors, and expression of a nociceptor-specific sodium channel, $Na_v1.8$. Methods include patch clamp recording from isolated sensory neurons followed by single-cell PCR for Ca channel splice variants.

The bottom line is that a variant of the N channel's exon 37 is expressed primarily on a subset of small nociceptive neurons. Exon37a replaces 37b, the usual variant, in a fraction of the N channels in some nociceptors, but this is less likely to happen in nonnociceptors. Specifically, 55% of neurons that respond to capsaicin express exon 37a, whereas only 20% of capsaicin-insensitive neurons do so. Cells expressing exon 37a are likely also to express $Na_v1.8$. Expression of exon 37a is undetectable in tissue other than sensory neurons.

What is the functional significance of exon 37a? The capsaicin-sensitive cells that express it have about 60% greater N channel current densities than those without it. When expressed in oocytes, calcium channels with the e37a variant are opened by somewhat smaller (10 mV) voltage stimuli than the e37b variant. We should suspect that further study will unveil additional function. Exon 37 encodes a short length (32 amino acids, 14 of which differ between the variants) of the C-terminal, cytoplasmic tail of the channel. Though this is a small fraction of the roughly 2000 amino acids in a calcium channel, the C-terminal region is critical for linking N channels to neuronal protein binding partners such as the ORL1 (nociceptin) receptor (Beedle et al., 2004). Such links create signaling complexes, perhaps including complexes central to neurotransmitter release (Augustine, 2001). For the purposes of drug discovery, questions will be (1) whether the small molecular difference between this channel and the more common splice variant is sufficient for the design of distinguishing drugs; (2) whether the low expression level of the e37a variant is biologically significant and thus worth inhib-